

PBB Homologs in Sera of Michigan Dairy Farmers and Michigan Chemical Workers

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In November, 1976 the Environmental Sciences Laboratory of the Mount Sinai School of Medicine, examined 1029 persons in Michigan for the potential health effects of exposure to PBB. Included in this group were 55 persons from Michigan Chemical Corporation and 237 farming families. Blood serum was analyzed for PBB.

FireMaster FF-1 contains several bromobiphenyl components, the major constituent being approximately 60% 2,4,4',2'-hexabromobiphenyl. Other PBB homologs identifiable as peaks by gas chromatography-mass spectrometry include two pentabromobiphenyl peaks, three additional hexabromobiphenyl peaks, and two heptabromobiphenyl peaks. The relative concentrations, with respect to the major hexabromobiphenyl peak, of these minor constituents (1-20%) of PBB were different for persons from Michigan Chemical Corporation and for farmers.

Penta-, hexa-, and hepta-bromobiphenyl components in serum samples analyzed from farming families from Michigan and from Michigan Chemical Corporation employees were compared with relative concentrations of these homologs in FireMaster mixture and in tissue and blood samples from rats fed FireMaster FF-1. Varying concentrations of these PBB components are attributed to different routes of exposure and the relative ease of metabolism and excretion of one pentabromobiphenyl component.

Commercial halogenated biphenyls inevitably consist of complex mixtures of many components with different degrees of halogenation and varying chlorine substitution isomers. Aroclor 1254 may contain as many as 70 individual chlorinated biphenyls, and Jensen and Sundström identified 60 individual components in Clophen A50 (1).

The brominated biphenyl mixtures are less complex, FireMaster BP-6 having nine prominent gas chromatographic peaks as determined by using electron capture detector (Fig. 1) whereas the comparable PCB mixture, Aroclor 1260, under similar conditions, shows at least 13 peaks by gas chromatography.

Many investigators have reported the marked preferential accumulation of higher chlorinated biphenyls in animals, which reflects the relative greater metabolism of lower chlorinated biphenyls. Among higher chlorinated biphenyls, those with appropriate substitution patterns are more susceptible to oxidation. For example, Aroclor 1254 ab-

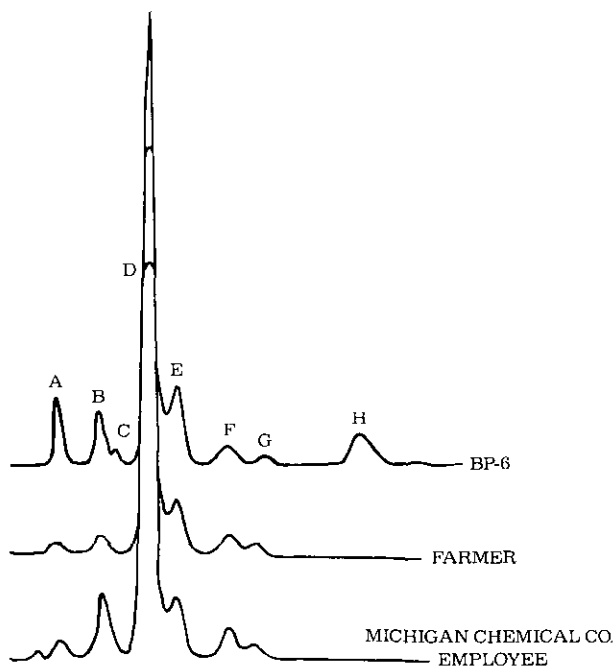


FIGURE 1. PBB peaks (GC-EC) in serum of a farmer and a Michigan Chemical Corporation employee.

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sorbed from the diet into adipose tissue of rats, after 4 weeks recovery, showed three major late-eluting peaks (2). After 30 weeks recovery, the first of these peaks was nil and the third diminished by half, both compared with the second peak.

Previous analytical studies (3, 4) have shown these three peaks to contain (in order) 3,4,2',4',5'-penta-, 3,4,2',3',4'-penta-, and 2,4,5,2',4',5'-hexa-; and 2,3,4,2',4',5'-hexachlorobiphenyls. These data suggest that 2,4,5,2',4',5'-hexachlorobiphenyl may be one of the more biologically persistent components of Aroclor 1254.

Similar patterns, denoted A and B by Kuratsune et al., for late-eluting PCB peaks were reported in adipose tissue of persons in Japan (5), and we also have observed two distinct patterns in serum of persons from the general population and in employees of a plant which used Aroclors extensively as electrical insulators.

PCBs in human adipose tissue are generally the higher chlorinated congeners, so that knowledge of the biological activity of these compounds has become important in assessing their potential human health effects.

Absorption and accumulation rates of PCBs have been studied, showing in general efficient absorption of most PCBs at doses below 100 mg/kg in rodents (6, 7). At higher doses, incomplete intestinal absorption allows fecal excretion, and occasionally even urinary excretion of certain unchanged PCBs. Recent investigations have explored the differential biochemical effects of pure PCB isomers.

The combined P-450/P-448 activity, reported for hepatic and cutaneous enzymes from rats exposed to PCBs, is now ascribed to combined discrete induction by particular components of the PCB mixture. Thus Goldstein et al. (9) and McKinney et al. (10) have shown that degree of chlorination is less important than the pattern of chlorine substitution of individual PCBs with relation to enzyme inducibility, lipophilicity, and toxicity. Sugiura (8) has studied accumulation and relative metabolism of tetrachlorobiphenyl. Individual tetra- and hexachlorobiphenyl isomers were shown to have quite distinct toxicity and enzyme inducibility, which could be correlated with chlorine substitution patterns, including degree of *ortho* and *para* substitution, molecular symmetry, and coplanarity of the two aromatic rings. Stonard has reported analogous results with three hexachlorobiphenyls (11, 12). PBBs have been less well studied, but similar differential biological effects are expected. It is notable that PBB absorption-elimination studies have shown fecal excretion in cows and in rats, even at relatively low (10 ppm) dietary levels (13, 14). For octabromobiphenyl, the major excretory route is

fecal (15), suggesting that absorption, accumulation and retention of PBBs may differ from that of PCBs, with excretory routes other than metabolic available. A recent study of absorption and excretion of 2,4,5,2',4',5'-hexabromobiphenyls has shown this compound, even at multiple doses of 30 mg/kg to be efficiently absorbed in the rat with less than 10% estimated to be excreted in a rat's lifetime (16). However, fecal and biliary excretion were noted for about 1% of the dose.

Studies of hepatic enzyme activity of the PBB FireMaster BP-6 show a spectrum of enzymatic effects attributable to both P-448 and P-450 enzymes, with a cytochrome P-450-carbon monoxide spectrum between that of phenobarbital and 3-methylcholanthrene-induced enzymes (17, 18). However, cytochrome P-448 is not a major component of induced mixed-function oxidases as it is with certain PCBs (19). By analogy with 2,4,5,2',4',5'-hexachlorobiphenyl, 2,4,5,2',4',5'-hexabromobiphenyl (the major component of FireMaster BP-6) would be predicted to have P-450 activity. As with the Aroclors, individual components of the PBB mixture may induce different microsomal enzyme systems, and induction by PBB may reflect the contribution of minor components of the mixture. In particular, the lack of P-448 induction suggests the absence of 3,4-substituted homologs.

Variabilities in tissue accumulation, toxicity, and metabolism of PBBs and the very marked differences of isomeric PCB components, have directed our attention to measurement of individual component peaks apparent in PBB mixtures from various biological sources. We here report the results of analyses of PBB components in sera from Michigan farmers and chemical workers.

Materials and Methods

Serum analytical methodology is described elsewhere (20). Since individual pure isomers were not available for calibration, analysis of component peaks was accomplished by calculating relative peak areas, setting the major 2,4,5,2',4',5'-hexabromobiphenyl peak at 100%. Because original calibration was based on the area of this peak from the weight of the entire mixture, the absolute values reported are useful only for comparative purposes, assuming a consistent error in all calculations.

For determination of PBB in rats, tissues and blood were obtained 4 days after administration of an 80 mg/kg dose of FireMaster FF-1 in corn oil by gavage to four 150g Sprague-Dawley male rats. Quantitative results are presented elsewhere, but adipose tissue concentrations of PBB were in the

range of 500 ppm. Tissues were prepared and analyzed essentially according to the method of Price (personal communication), except that tissues were ground with hexane in a Duall 23 grinder to extract PBBs. PBB homolog recovery from rendered chicken fat was identical to that of the original mixture, and PBB recoveries were quantitative (92–107%).

Results and Discussion

Variation in PBB homolog content of human blood serum was readily observed at high PBB concentrations (> 20 ppb in serum, Fig. 2). At lower concentrations, peak A could be obscured by concomitant PCB peaks. Among both chemical workers and farmers, peak A was greatly reduced, proportionally less among farmers than among chemical workers (Table 1). Several chemical workers with high serum PBB levels were former PBB production workers with presumably intense exposures. Farmers, however, had important dietary exposure to PBB, which could have undergone partial metabolism in the animal food source. Further, the FireMaster FF-1 lot which caused contamination of livestock feed had a smaller proportion of peak B than that in some lots of FireMaster BP-6 (e.g., Figure 2) (21). However, peak B in farmers' sera was still smaller than that in the appropriate FireMaster FF-1. In rats fed this same lot of FireMaster FF-1, peak B was proportionally greater than in farmers' sera, and again comparable to that in sera of Michigan Chemical employees (Table 1). In these rats, recovered 4 days from a single PBB dose, peak A was markedly reduced (Fig. 2, Table 1), while peak B remained at a concentration similar to that in the original PBB mixture. Peaks A and B, though both pentabromobiphenyls, may be expected to have quite different rates of metabolism, depending on the bromine substitution pattern. Both peaks A and B were significantly lower among farmers than among chemical workers, and peak A was significantly reduced from that in FireMaster PB-6 in farmers, workers, and rats (Table 1, Fig. 3).

In contrast, the heptabromobiphenyl peak H was observed at comparably low concentrations, approximately 1% or less of the major peak, compared with 22% in the original mixture. In rats, the 2,3,4,5,2',4',5'-heptabromobiphenyl is readily absorbed along with other PBB components and persists unchanged for long periods (22). Absorption-excretion patterns of heptabromobiphenyls in man and in cattle are apparently different from the rat, at least over the long term (23). (Willet et al., in press).

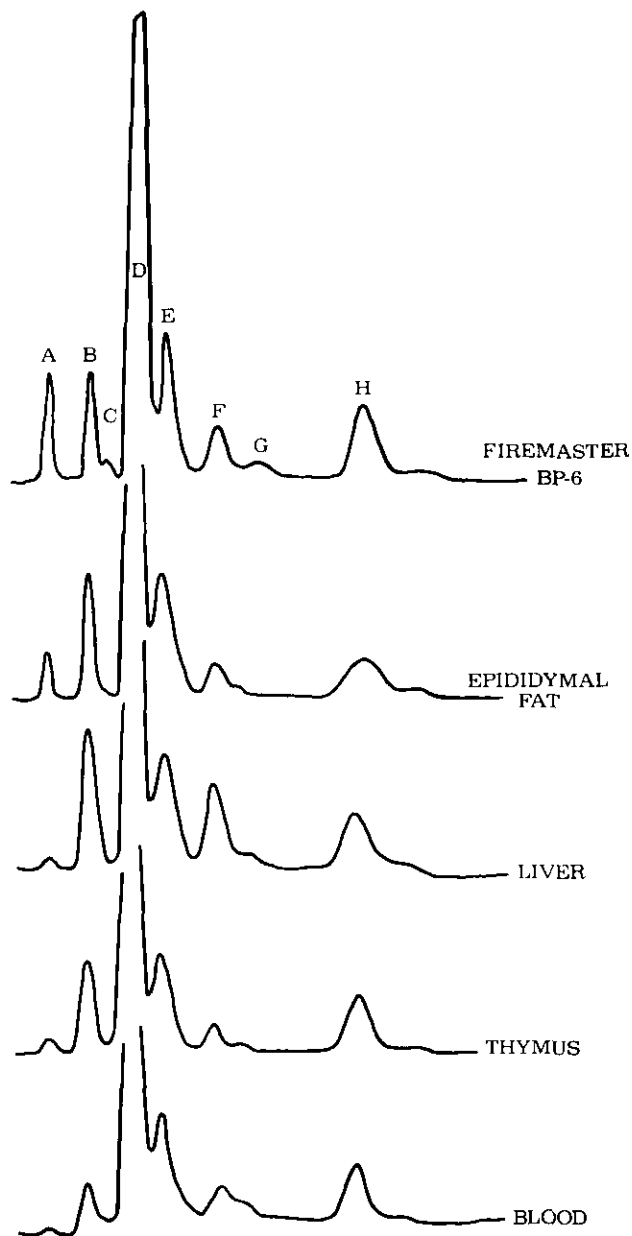


FIGURE 2. PBB homologs in blood and tissues of rats 4 days following 80 mg/kg dose.

The significance of varying PBB homolog content of the human body burden awaits toxicological assessment of individual PBB compounds. While 2,4,5,2',4',5'-hexabromobiphenyl and the heptabromobiphenyl homolog can singly cause persistent induced hepatic enzymes, the activity of the lower brominated compounds, including unidentified contaminants such as bromonaphthalenes, has not been determined. The experiments reported by

Table 1. Relative area of PBB homolog peaks (peak D = 100).

Peak	Component	Peak area ratios ^a			
		FireMaster BP-6	Serum from Michigan Chemical Corp. employees	Serum from dairy farmers	Serum from rats (4 days after 80 mg/kg dose)
A	Pentabromobiphenyl	7	5 ± 3	1 ± 1	< 1
B	Pentabromobiphenyl	12	13 ± 6	3 ± 1	9 ± 1
C	Hexabromobiphenyl	2	0.2	—	—
D	2,4,5,2',4',5'-Hexabromobiphenyl	100	100	100	100
E	Hexabromobiphenyl	24	13 ± 6	13 ± 4	24 ± 1
F	Hexabromobiphenyl	7	5 ± 4	6 ± 4	9 ± 2
G	Heptabromobiphenyl	4	0 - 3	< 1 - 5	5 ± 6
H	2,3,4,5,2',4',5'-Heptabromobiphenyl	19	2 ± 3	2 ± 2	22 ± 2
I	Heptabromobiphenyl	1	< 1	< 1	< 1

^a Peak areas ratios, expressed as mean ± standard deviation, were calculated for 24 chemical workers (serum PBB > 10 ppb) and 37 farmers (≥ 15 ppb). The means of the two groups were significantly different ($p < 0.001$) for peaks A and B.

Safe et al. (24) along with previous studies of the so-called nonpolar fraction of PBB by Hass et al. (25) and Kimbrough et al. (26) do not preclude the possibility of the lower brominated compounds, including the pentabromobiphenyls, contributing to the differential toxicity of various components of PBB mixtures.

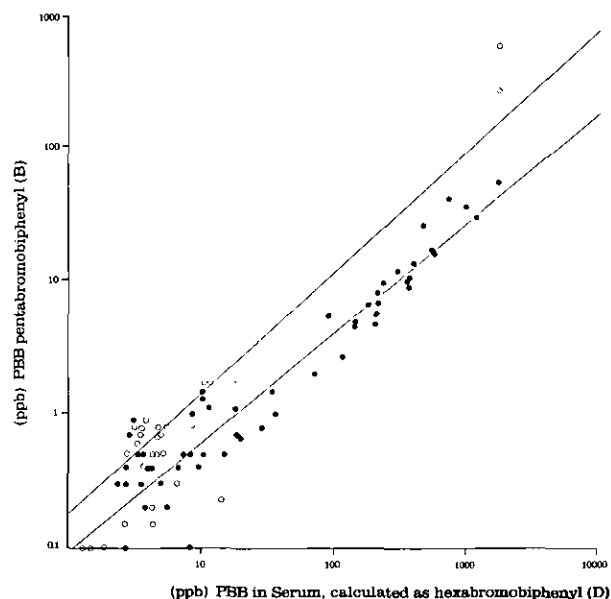


FIGURE 3. Concentration of pentabromobiphenyl (B) in sera of (●) farmers and (○) Michigan Chemical Corporation workers relative to total PBB.

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